U.S. Fish and Wildlife Service
Reynoldsburg, Ohio Field Office
6950-H Americana Parkway
Reynoldsburg, OH 43068
614-469-6923

Herbicide Concentrations in Waters of Northwestern Ohio

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By: William J. Kurey, Biologist

ABSTRACT

New and relatively inexpensive enzyme linked immunosorbent assay (ELISA) kits in the 96 well plate format were used to determine triazines and alachlor concentrations in waters of northwestern Ohio. Alachlor and related herbicides reached peak concentrations in June, the highest being 36.5 ug/l in Muddy Creek. The alachlor concentrations probably result in part from detection of other herbicides as well as alachlor by the ELISA test, such as metolachlor. Triazines also peaked in June but with the highest concentration being only 4.7 ug/l, also in Muddy Creek. Herbicide concentrations measured in this study are probably below actual maximums experienced during and shortly after storms. Herbicide concentrations in waters of Ottawa National Wildlife Refuge were generally lower than other area waters and probably don't represent a significant hazard to biota, or at most a temporary hazard. Refuge managers should be aware that heavy rains previous (within about 10 days) to pumping water into refuge units will have increased the amount of herbicides in the water being pumped.

INTRODUCTION

One purpose of this study was to assess the utility of enzyme linked immunosorbent assay (ELISA) technology for field offices of the U.S. Fish and Wildlife Service. ELISA tests were used to measure the levels of two common agricultural herbicides in the surface waters of the Ottawa National Wildlife Refuge (NWR) and other waters of northwestern Ohio. Atrazine and alachlor were the herbicides chosen for this study because they are known to be used in the area in large quantities. In 1982 these two herbicides were the most heavily used in the country with atrazine comprising 17.3% of the total herbicide poundage and alachlor 19.1% (Pratt et al. 1988).

Ohio is part of the corn belt and agricultural herbicides used with corn reach higher concentrations in rivers of northwestern Ohio than anywhere else in North America with combined concentrations occasionally exceeding 250 ug/l (Baker et al. 1981). The concentration pattern in streams is predictable with peak concentrations in early summer during storm runoff events following corn and soybean planting. Krieger et al. (1988) suggested that much higher combined concentrations of herbicides are likely in headwater streams where edge-of-field conditions exist. Atrazine, used on corn, and alachlor (Lasso), used on corn and soybeans are major herbicides in surface waters, both having recorded peaks of about 100 ug/l in smaller tributaries of Lake Erie (Baker 1988). These herbicide concentrations are high enough to affect some aquatic plants although no long lasting effects on the composition or production of aquatic plant communities have been noticed (Krieger 1989).

Atrazine is a commonly used preemergent herbicide for the control of weeds in corn and sorghum crops. Potential residual build-up and migration from the soil into groundwater has caused concern about its widespread use. It has been detected in groundwater more often and at higher concentrations than any other pesticide. Traces of atrazine have also been found in rainwater samples taken from a 23 state area of the Midwest and Northeast indicating that it can vaporize during application and return in rain and fog (Uhlenbrock 1992, Wu 1981). Atrazine is the most frequently detected pesticide in both ground and surface waters of the Midwest (Uhlenbrock 1992). Surface water concentrations of alachlor are high throughout the year in the cornbelt with the annual drinking water exposure often between 1 and 5 ug/l. The short term concentration of alachlor may be several orders of magnitude higher. Atrazine is listed as a Class C carcinogen, ie. a possible carcinogen. Alachlor is also a carcinogen.

The Environmental Contaminants branch of the U.S. Fish and Wildlife Service (FWS) relies primarily on gas chromatography and atomic absorption spectrometry to determine the environmental levels of contaminants in most media. These are very accurate and precise methods for the job but they are

too expensive to be used more than sparingly in most applications. An alternate type of analysis, the immunoassay, has recently become available for some substances. Immunoassay technology has been used for testing in the medical field for about the last 20 year, the Food and Drug Administration having approved 150 immunoassay diagnostic tests. Only in recent years has this technology been applied to analysis of environmental samples (Carter 1992).

The lowest cost of an analysis for atrazine and alachlor in water (1 sample, gas chromatography) through the Patuxent Analytical Control Facility (part of the FWS) in 1990 was about $196. Using ELISA the cost of doing both analyses on a single water sample was less than $7 (2 wells based on the 1990 cost of $300 apiece for the 96 well plate kits only). The capital costs for the ELISA analyses were the strip reader at $1875 in 1991, adjustable pipette and tips in 1991 at $219.50 and $43.50 respectively, and 2 cooking timers at $16 for a total cost of about $2154. This investment in equipment would be paid for in the cost of only 11 gas chromatography analyses at the cost of $196 each.

From one manufacturer in 1990 the list of commercially available ELISA kits in the plate format included triazines, alachlor, aldicarb, benomyl, 2,4-D, metalaxyl, and methoprene. These, as well as carbofuran and cyclodienes kits, were also available in the less precise tube format. A test for PCBs in both soil and water (tube format), petroleum in soil (tube format), and pentachlorophenol in soil (tube format) have recently became available and the list of tests commercially available will undoubtedly continue to grow. Assay kits for aflatoxins are available from several companies and Vanderlaan et al. (1988) predicted a test for dioxin that would cost $10 per assay and have a detection limit of about 1 ng.

Apparent recoveries of triazine herbicides in the range from 0.2 to 2.0 ug/l were comparable between gas chromatography/mass spectrometry (GC/MS) and ELISA from natural water and spiked water (Thurman et al. 1990). Significant cross reactivity between triazine herbicides such as atrazine, propazine, and simazine made the ELISA test a reliable screening method for environmentally significant concentrations of the triazine herbicides as a class. Cyanazine, triazine breakdown products, and some other triazines were not detected by the ELISA test (Thurman et al. 1990).

The alachlor plate kit cross reacts with metolachlor, matalaxyl, and did not differentiate between the various alachlor compounds. There is also evidence that the alachlor test detects alachlor degradation products to a high degree, especially ethane sulfonic acid, giving false positive results for alachlor (Baker 1992, unpublished). Results of toxicity tests on ethane sulfonic acid are not yet available.

METHODS

Water samples were collected from a number of water bodies in northwest Ohio at various times in 1991 (see Table 1). Field trips to the sampling sites were scheduled as the author's other workload allowed. Samples were collected either directly from the water by inserting a cleaned 500 ml. screw top glass jar by hand or with an alpha bottle sampler. Collection jars and lids were cleaned prior to each use by washing with hot soapy tap water, soaking and then rinsing in clean tap water, and then rinsing the inside of the jar with acetone. All samples were collected from near the water surface, generally from at or near the shoreline. Water samples were refrigerated until testing and all tests were completed within 3 days of sampling. Secchi disk depth measurements were occasionally taken from some stream locations and ranged from 6 to 23 inches and averaged about 10 inches.

Enzyme linked immunosorbent assay kits ("EnviroGuard" plate kits) for triazine herbicides and alachlor were purchased from Millipore Corporation. Kit directions were followed during the analyses except that 80 ul instead of 40

ul of chromogen was added in step 8, and all materials were delivered to the plate wells using a Gilson "Pipetman" P-100 pipette which is continuously adjustable between 0 and 100 ul. in 1 ul. increments. Rainin RT 20 disposable pipette tips were changed after each delivery of a sample or standard and after all deliveries of a given reagent were completed. Locally purchased 1 hour cooking timers were used to time the required incubation periods. The 2 incubations periods in the 96 well plate format protocol are 1 hour and 30 minutes making the tests relatively fast. Clear packing tape was found to perform better than Parafilm in sealing the wells during incubation. No orbital mixer was available during the incubation period and tap water with no special equipment was used to wash the wells. All ELISA kits were used within their specified shelf life period.

The manufacturer's directions specified that 2 drops or 40 ul of chromogen be used in step 8 of the protocol but in previous steps 2 drops had been defined as 80 ul. Questions to the manufacturer about this apparent error were originally answered by affirming 80 ul as the correct amount and this was the amount used in this study. Representatives of Millipore Corp. have subsequently stated that 40 ul was the intended quantity and that the difference in defining a drop was due to the difference in surface tension of chromogen. They also stated that the amount was not critical as long as the quantity was uniform throughout the test. In a published paper by Stearman and Adams (1992), using the same triazine kit, 80 ul of chromogen was also used.

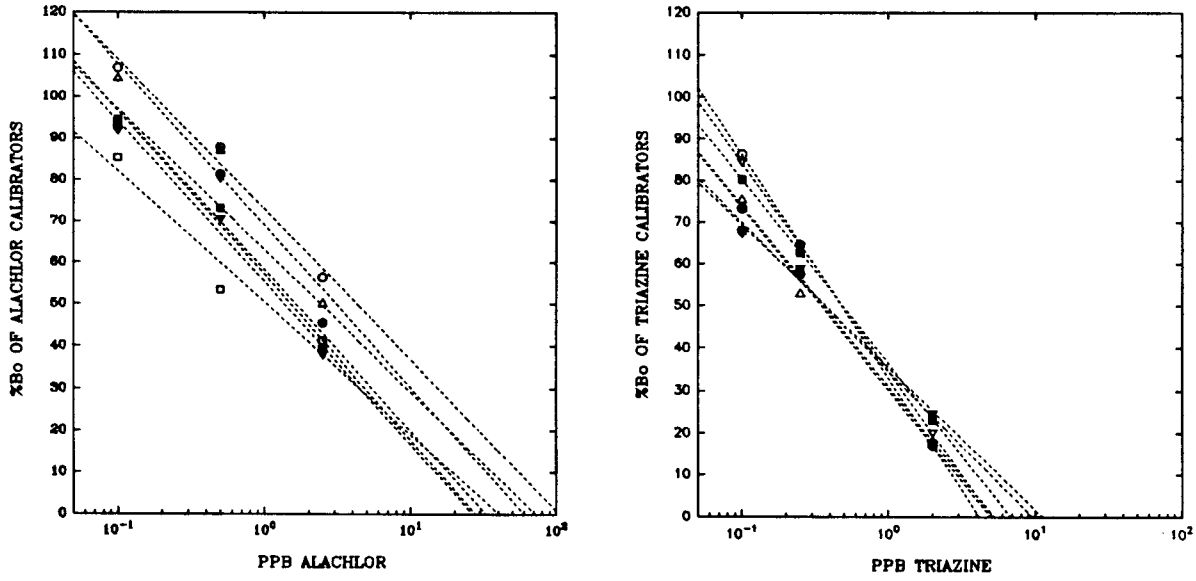

Fig. 1. Regressions of calibration solutions for various triazine and alachlor analyses. These lines were used to determine the concentration of herbicide in a sample.

Table 1. Descriptions of water sampling locations in northwestern Ohio, 1991.

NAME OF SAMPLING SITE	LOCATION OF SAMPLING SITE
MAUMEE R.	The Maumee River at Waterville, OH from a park on the west side south of the Rt. 64 bridge
MSU-8B	Ottawa NWR Moist Soil Unit 8B near the headquarters building
CRANE CK.	Ottawa NWR from the middle of the Stanger Rd. bridge
MSU-5	Ottawa NWR Moist Soil Unit 5 from the area of the pump
POOL-3	Ottawa NWR Pool-3 near the pump
L. ERIE (CEDAR PT. E.)	Lake Erie shore on the east side of the Cedar Point NWR (Ottawa NWR) near the pump
L. ERIE (CEDAR PT. W.)	Lake Erie shore on the west side of the Cedar Point NWR (Ottawa NWR) near the pump
CEDAR PT. E.	Cedar Point NWR (Ottawa NWR) on the east side near the pump
CEDAR PT. W.	Cedar Point NWR (Ottawa NWR) on the west side near the pump
DARBY (POOL)	Ottawa NWR Darby Unit Pool-4 near the pump
DARBY (L. ERIE)	Lake Erie shore at Pool-4 of the Ottawa NWR Darby Unit
DARBY (CANAL)	Ottawa NWR Darby Unit near the pump on the canal from Lacarpe Ck.
TOUSSAINT R.	The Toussaint River from the north end of the Rt. 2 bridge
PORTAGE R.	The Portage River from the north end of the Rt. 2 bridge
MUDDY CK.	Muddy Creek from the north end of the Rt. 53 bridge
SANDUSKY R.	The west side of the Sandusky River from the picnic area at Wolf Creek State Park

The 96 well plate kits were broken into 12 well strips or smaller for this study. Each field day 12 or 13 water samples were collected necessitating the use of about 3 strips each for triazine and alachlor tests. The several strips were held in the original plate frame during development. The samples were read with a Bio-Tek strip reader Model EL301 photometer using a 450 nanometer wavelength filter. New sets of water blanks, negative controls, and calibrators were used for each set of analyses and also each time a new kit was used in a set of analyses. To avoid using a good well for a water blank, previously used wells were substituted in the strip carrier just prior to reading. Blanks, controls, calibrators, and samples were run in duplicate at least and results were averaged for each sample.

It should be noted that since these analyses were completed the manufacturers of ELISA kits have changed the format of the 96 well plates and the 12 well strips. The smallest number of wells that can be developed with the new format is 12 because the square plate that holds the 12 well strips no longer has a lattice work bottom to hold odd numbers of wells- the new plate is an empty rectangle. The new 12 well strips are thickly joined at the base and side making their separation into shorter units to save wells practically impossible. The 12 well strip has also been modified by producing small nubs near the center of the base requiring that they be carved off with a knife, a new strip carrier for the photometer be purchased, or the old style strip carrier be modified.

Optical density (absorbance) readings were converted to %Bo following kit directions. The calibrators were plotted (%Bo versus parts per billion of herbicide) and a linear regression was carried out in a semi-log format using Jandel Sigma Plot graphics software (see Fig. 1). This was done to more easily and accurately obtain the x and y axis coordinates where the regression line crossed the axes. These points were then used to construct a similar graph on 2 cycle semi-log graph paper. Graph paper provided more graduations in the grid than did the graphics software. Using the %Bo and the regression line the corresponding parts per billion of herbicide residue was manually obtained for each sample.

As a check on the concentrations derived manually a spreadsheet in Lotus 123 was devised to automatically derive the %Bo, standard regression line, and concentration from optical density data. Doing some of the calculations manually is a good check but a computerized system is essential for efficient processing of large numbers of samples. Sample concentrations in this study were computer calculated for greater precision.

RESULTS

Data sets are incomplete for several 1991 sampling sites due to discontinued or late initiation of sampling (see Table 2). An effort was made to get the best 12 or 13 sampling locations, the maximum number of locations that was reasonable for a 1 day field trip. Although the sampling times are fairly evenly spaced through the sampling period, there is a gap between August and October, no samples having been collected in September.

The results of all 1991 analyses are reported in Table 1. These data were then graphed in Figures 4 through 9. For graphic analysis the waters of northwestern Ohio were grouped into three categories: waters on the Ottawa NWR (mainly in diked pools); Lake Erie; and streams and rivers. The patterns that resulted were distinctly different for the triazines and alachlor. The patterns were also different within each herbicide when the different types of water systems were considered.

The levels of alachlor in all waters was generally much higher than the triazines throughout the sampling period except for several data points in the October 23 sampling. Both herbicides show a marked peak concentration in June with subsequent declines. The high levels of alachlor may have been the

result of the test kit detecting both alachlor and metolachlor.

Baker and Richards (1989) summary of atrazine, metolachlor, and alachlor concentrations for waters of the Maumee River and Sandusky River between 1983 and 1988, which were the result of gas chromatography analysis, show atrazine levels to be much higher than alachlor. The concentration of metolachlor was also about twice that of alachlor. The high concentrations of alachlor relative to triazines in our 1991 study is probably the result of the test kit for alachlor detecting both alachlor and metolachlor. With this understanding the 1991 data for alachlor is in much better agreement with those of Baker and Richards (1989). Baker and Richards (1989) summary of peak concentrations for atrazine in the Maumee River and Sandusky River were similar to levels encountered in our 1991 study.

The width of the graphed peaks for triazines were wider than for alachlor indicating that high concentrations of triazines lasted for longer periods of time (see Figs. 4-9). Triazine concentrations in streams and rivers and Lake Erie were higher during the sampling period than the Ottawa NWR waters, generally less than 1 ppb (see Figs. 7-9). The alachlor concentrations in pools on the Ottawa NWR showed a steady decline from the first day of sampling, another distinct pattern (see Fig. 4). A single exposure at the time of pumping with no further inputs would be expected to show such a pattern. Triazine concentrations in Ottawa NWR pools and moist soil units were uniformly low.

Crane Creek, a stream on the Ottawa NWR, displayed the alachlor pattern typical of other streams and rivers but at lower concentrations. Alachlor concentration patterns in streams and rivers showed a plateau before the final decline which was not present in the Lake Erie samples or other Ottawa NWR samples (see Figs. 4-6).

The alachlor as well as triazine concentrations in Lake Erie at the Darby Unit of the Ottawa NWR were much lower than on either side of the Cedar Point Unit of the refuge which is adjacent to Maumee Bay and about 15 miles distant. Perhaps this indicates that the Maumee River is the major source of herbicide loading to this region of the Lake and the low concentrations at the Darby Unit are the result of dilution.

Virtually all atrazine in surface waters is in the dissolved phase (Bodo 1991). Atrazine and its phytotoxic metabolite desethylatrazine were common in surface waters of Ontario in areas were atrazine was used. Desisopropylatrazine, a less toxic degradation product, was a less significant compound. Studies of small watersheds and large rivers have shown that concentrations of atrazine increase with storm water runoff events during the summer spray season (Bodo 1991). Bodo (1991) observed surface water concentrations of atrazine in southern Ontario to be highest in the spring and summer application period.

Huckins et al. (1986) gave the topsoil half-life of atrazine as 48 to 71 days and the edge-of-field concentration as 1.03 ppm (mg/l) in runoff. In laboratory tests of sediment and water mixtures they found that 4.9% of the atrazine was in the sediment and 95.1% was dissolved in the water. In microcosms atrazine was highest in tissues of algae, macrophytes and midge larvae (adult midge had 1/3 the residues of larvae) (Huckins et al. 1986).

It is possible to lose 10% of an applied pesticide during a single storm event if it has not been incorporated into the soil (Wauchope 1978). In 1989, the paired watershed demonstration project between the Bayou (conservation watershed) and the Lacarpe (control watershed) watersheds in Ottawa County, Ohio showed the percent of Atrazine in runoff (runoff:application) to be 5.9% and 18.1% in the respective watersheds. These figures demonstrated that agricultural practices can significantly affect the amount of herbicide entering surface waters.

The triazine concentrations in northwest Ohio surface waters in 1991 followed a pattern similar to those observed in Ontario and previously in northwestern Ohio (Figs. 7-9). Atrazine concentrations normally decline through autumn but residues persist into the following year (Bodo 1991). Larger watersheds tend to have less annual variability in concentrations and lower peak pesticide concentrations but the duration of intermediate concentrations is increased (Baker and Richards 1989). This could mean that biota in a large river, such as the Maumee River and perhaps Maumee Bay, suffer any effects of herbicide contamination to a greater degree than smaller streams due to the prolonged exposure.

Krieger (1984) in a study of Old Woman Creek Estuary in Erie County Ohio found that water residues of 5 herbicides in 1983 reached a maximum total concentration of 28 ug/l. Atrazine alone reached 11 ug/l. In Honey Creek, June 1981, Baker et al. (1981) found that concentrations of atrazine persisted at levels greater than 10 ug/l for 24 days after a storm in which the atrazine peak concentration was 89 ug/l. This same storm caused alachlor and metolachlor levels greater than 10 ug/l for over 8 days. In May of 1982 herbicide residues in water from upper Sandusky Bay totaled 97.6 ug/l with atrazine at 18 ug/l, alachlor at 25 ug/l, and metolachlor at 41 ug/l (Krieger 1984).

Atrazine residues in rainwater from the Chesapeake Bay area ranged from .003 to 2.19 ug/l in a study by Wu (1981), with winter rain residues unexpectedly high (.003 to .97 ug/l). In that study atrazine residues in the Rhode River estuary generally ranged much lower, .006 to .190 ug/l, and high winter rain concentrations did not affect estuarine water concentrations. High atrazine levels in winter rain suggested that once atrazine is released into the atmosphere it can remain for long periods of time and travel great distances (Wu 1981). Concentrations in estuarine water declined from October until the spring spraying.

Wu et al. (1980) found that water surface microlayer enrichment of atrazine varied from none to 110x, 46% of the 65 samples in the study with an enrichment factor of 5x or less. Actual concentrations in the water surface microlayer ranged from 0.01 ug/l (water column .003 ug/l) to 0.59 ug/l (water column 0.19 ug/l). Such enrichment has also been noted for many other airborne pollutants. Surface microlayer enrichment was highest for atrazine between mid September and late October. The monthly water column concentration patterns of atrazine found by Wu (1980) were much lower and apparently lacked the distinct June and July peak characteristic of northwest Ohio waters.

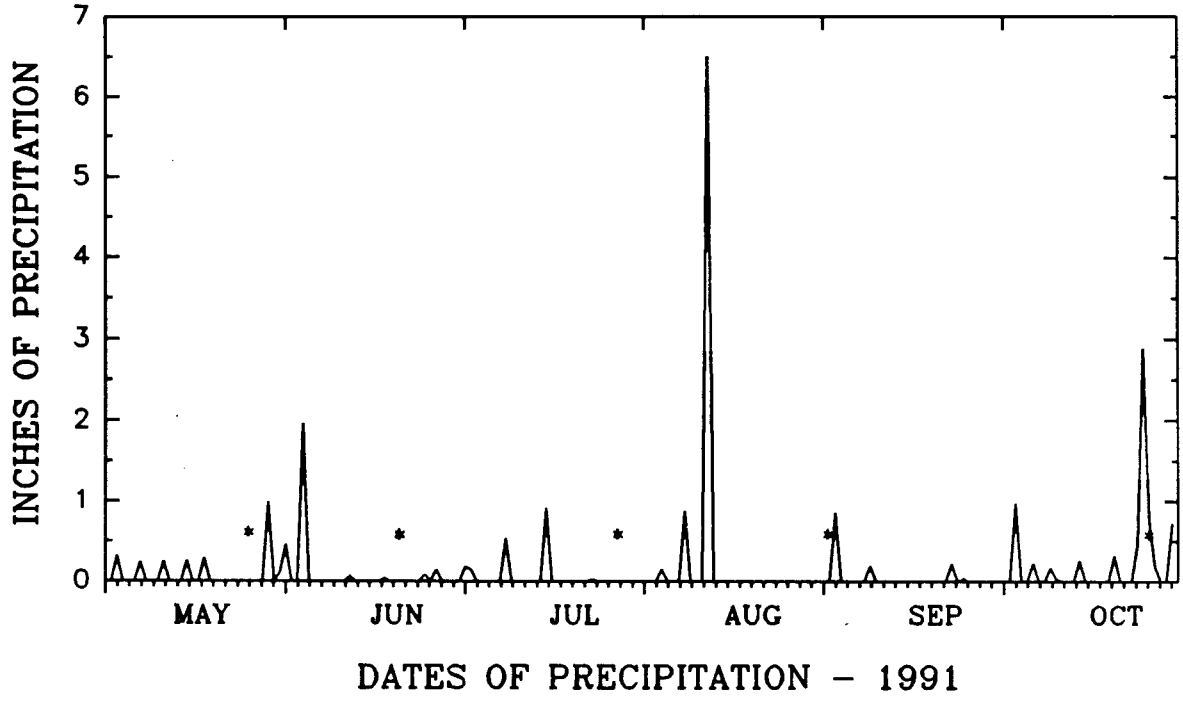

Fig. 2. Precipitation at the Ottawa National Wildlife Refuge rain gauge during the sampling period in 1991. Dates on which water samples were collected are indicated with an asterisk.

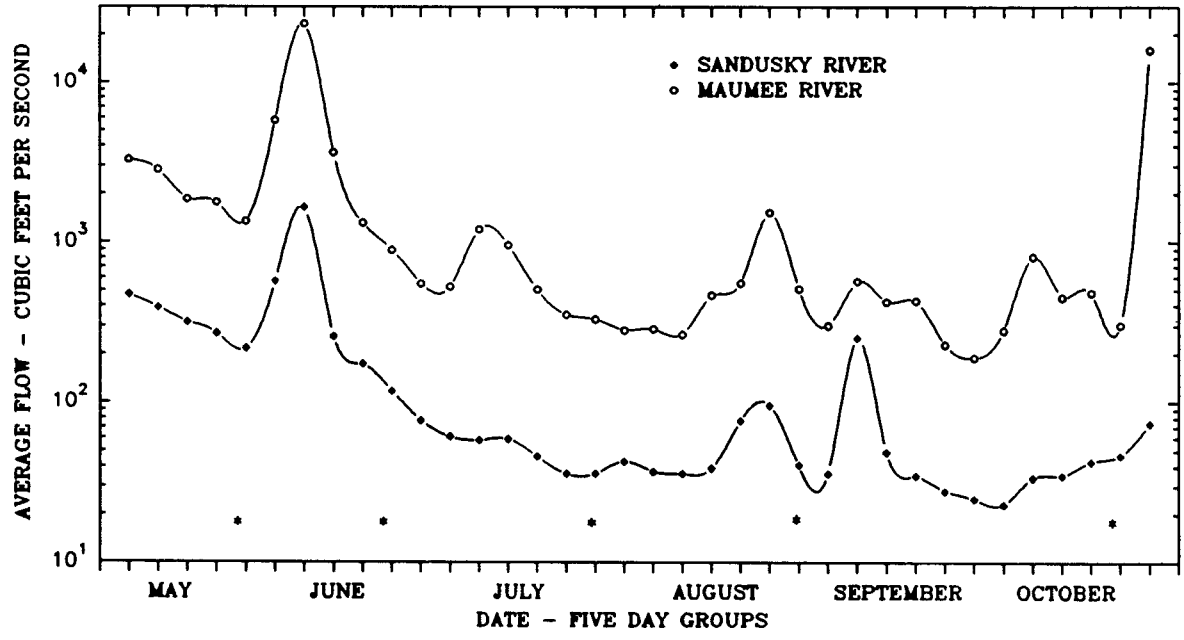

Fig 3. Five day average flows in the Sandusky River and the Maumee River in 1991. Asterisks indicate sampling dates.

Fig. 4. Alachlor residues in surface waters of the Ottawa National Wildlife Refuge (NWR) in 1991.

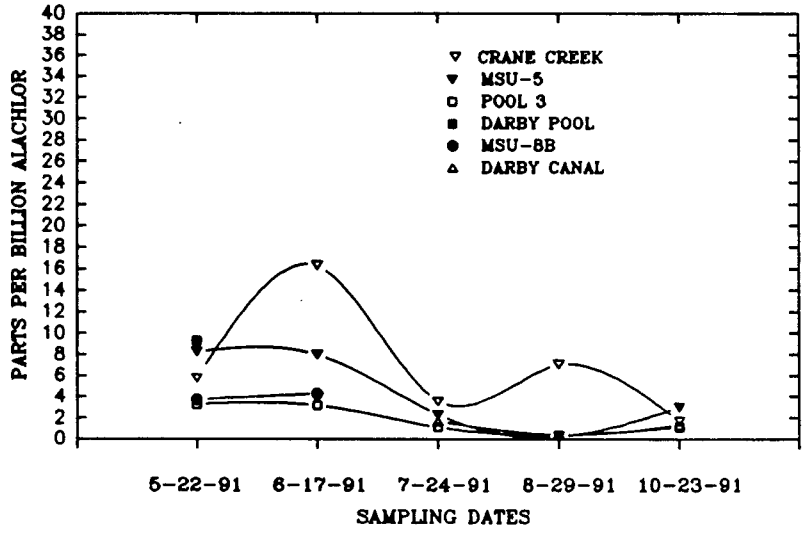

Fig. 5. Alachlor residues in Lake Erie water off the shores of Ottawa NWR in 1991.

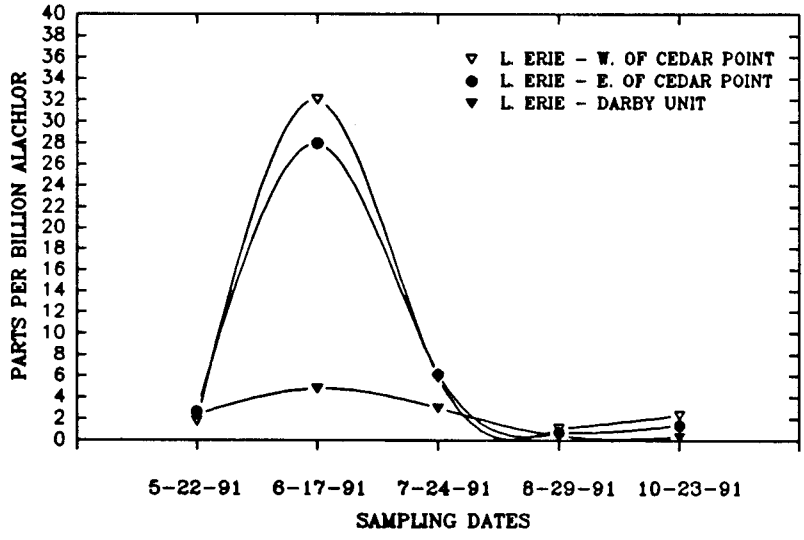

Fig. 6. Alachlor residues in surface water of streams and rivers of northwestern Ohio in 1991.

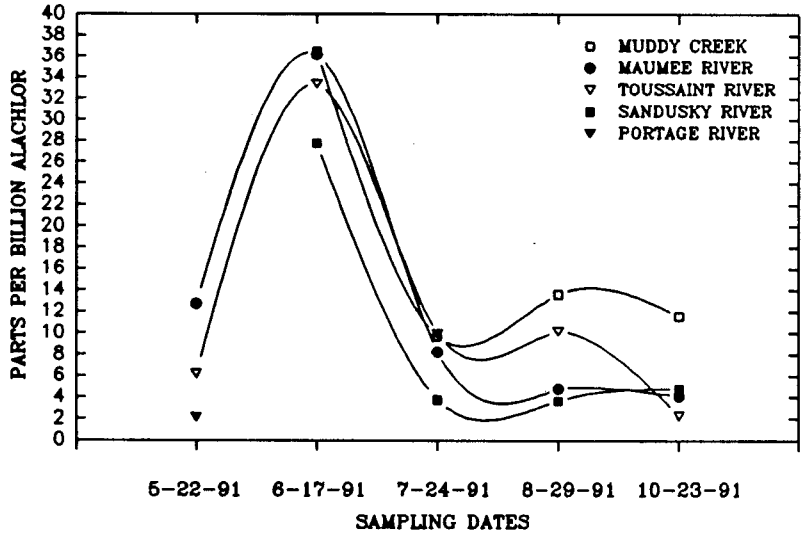

Fig. 7. Triazine residues in surface waters of the Ottawa NWR in 1991.

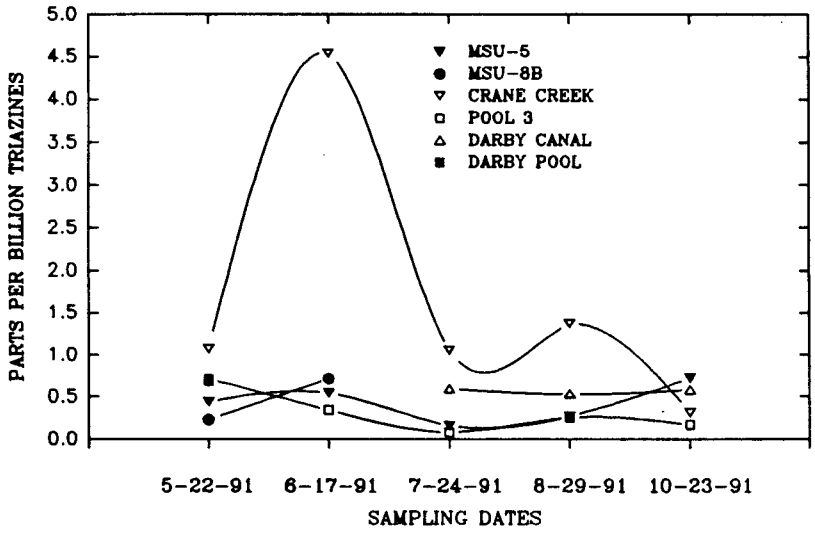

Fig. 8. Triazine residues in Lake Erie water off the shores of Ottawa NWR in 1991.

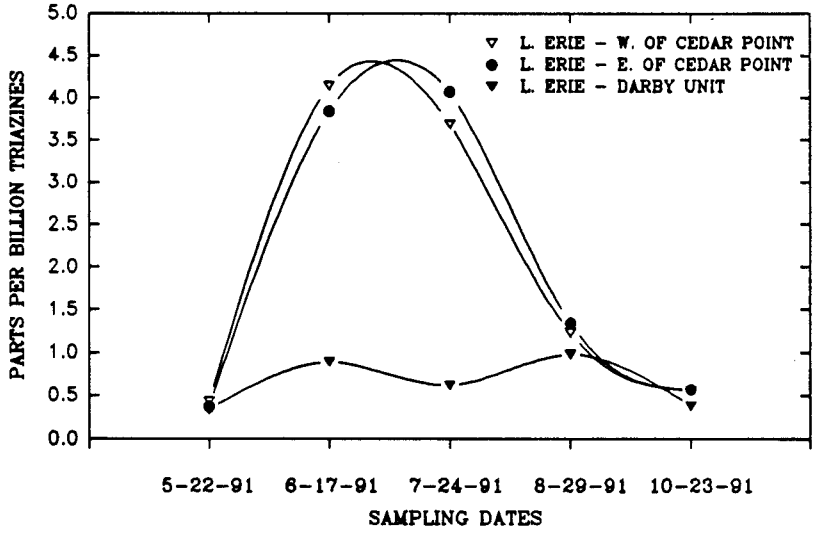

Fig. 9. Triazine residues in surface water of streams and rivers of northwestern Ohio in 1991.

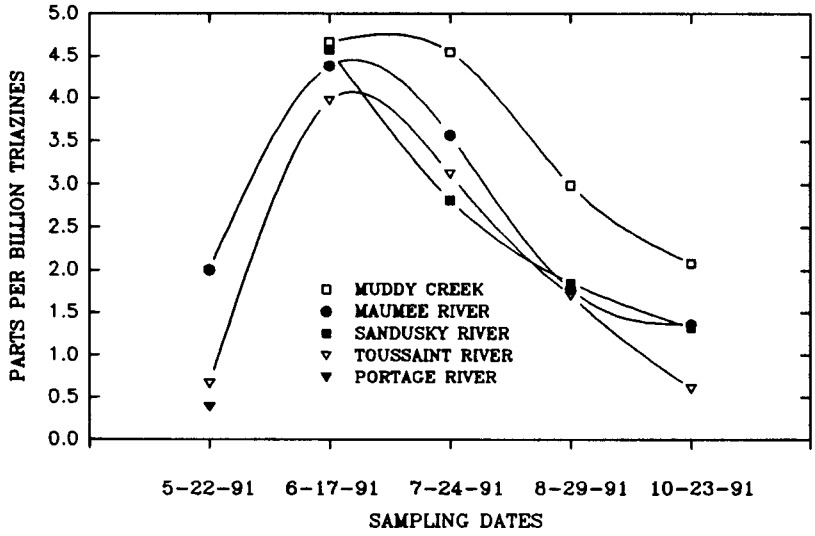

Table 2. Results of ELISA tests on water from various northwest Ohio locations for alachlor (ALA) and triazine (TRI) herbicides. The concentration units are parts per billion.

	5-22-91		6-17-91		7-24-91		8-29-91		10-23-91	
LOCATION	ALA	TRI	ALA	TRI	ALA	TRI	ALA	TRI	ALA	TRI
MAUMEE R.	12.72	2.00	36.17	4.38	8.26	3.57	4.83	1.78	4.11	1.36
MSU-8B	3.75	0.23	4.28	0.71	-	-	-	-	-	-
CRANE CK.	5.76	1.07	16.34	4.54	3.54	1.05	7.09	1.37	1.68	0.31
MSU-5	8.28	0.44	8.00	0.54	2.29	0.16	0.24	0.27	3.01	0.72
POOL-3	3.29	0.71	3.18	0.34	1.11	0.08	0.39	0.25	1.14	0.17
L. ERIE (CEDAR PT. E.)	2.65	0.37	27.98	3.84	6.19	4.07	0.74	1.34	1.40	0.57
L. ERIE (CEDAR PT. W.)	1.77	0.43	32.07	4.14	5.88	3.69	1.13	1.23	2.33	0.56
CEDAR PT. E.	3.89	0.11	-	-	-	-	-	-	-	-
CEDAR PT. W.	10.30	0.70	31.56	4.38	-	-	-	-	-	-
DARBY (POOL)	9.30	0.68	-	-	-	-	-	-	-	-
DARBY (L. ERIE)	2.39	0.34	4.82	0.89	3.01	0.62	0.47	0.98	0.34	0.38
DARBY (CANAL)	-	-	-	-	1.67	0.59	0.44	0.53	1.31	0.58
TOUSSAINT R.	6.19	0.66	33.38	3.97	9.98	3.12	10.20	1.70	2.27	0.60
PORTAGE R.	2.11	0.38	-	-	-	-	-	-	-	-
MUDDY CK.	-	-	36.46	4.67	9.66	4.55	13.62	2.99	11.59	2.08
SANDUSKY R.	-	-	27.75	4.57	3.75	2.82	3.66	1.85	4.76	1.32

DISCUSSION

Atrazine is one of the best studied herbicides regarding environmental effects. The environmental impacts of most other herbicides on non-target systems have been little studied. Triazines (including atrazine) and acetanilides (including alachlor) have different toxic mechanisms in plants. Triazines directly inhibit photosynthesis (Moreland 1980) whereas the acetanilides interfere with starch mobilization, lipid synthesis, and damage membranes (Ebert 1980). Effects of herbicides on heterotrophic components of the aquatic community may result from alterations of the food chain and other community level interactions (Pratt et al. 1988).

Atrazine can also affect metabolic function at lower concentrations than required to affect community structure. In microbial communities the most sensitive measures of toxic stress from atrazine were oxygen production and magnesium and calcium metabolism. Species richness and biomass were less sensitive to atrazine (Pratt et al. 1988). Species numbers decreased in protozoan communities at 337 ug/l atrazine and the colonization rate was reduced at 3.2 ug/l. Oxygen, magnesium, and calcium metabolism were affected at around 17.9 ug/l with the calculated LOEC given as 32 ug/l (Pratt et al. 1988). Atrazine also inhibited ammonium oxidation and increased nitrite oxidation by nitrifying bacteria in aquatic systems (Gadkari 1988), but this occurred at the environmentally unlikely concentration of 1 ppm.

Hartman and Martin (1985) found that atrazine did not affect the growth of Lemna minor in acute toxicity tests below a threshold of about 100 ug/l, either with or without suspended sediment. The EC50's (48 hrs.) of alachlor with L. minor were 10.1 ug/l and 14.5 ug/l with and without suspended sediment respectively. Atrazine significantly inhibited growth of Potamogeton pectinatus at concentrations as low as 100 ug/l. Alachlor's effect on P. pectinatus growth are manifested between 1.0 mg/l (no effect) and 10 mg/l (growth inhibition) with growth being slightly stimulated at about 0.1 mg/l. The effect of suspended sediment on toxicity of these herbicides was not important in these tests. Stay et al. (1989) also found that the lowest observed effective concentration (LOEC) of atrazine in Leffler microcosms was about 100 ug/l. The Leffler microcosms were 1 liter beakers of water inoculated with a mixed culture of algae and invertebrates developed from a natural community.

The concentrations at which atrazine demonstrated harmful effects to wildcelery (Valisneria americana) were 3.2 to 12 ug/l over a 7 week period (Correl and Wu 1982). These low concentrations are within or near the ranges seen in waters of northwestern Ohio. The original western Lake Erie marshes contained beds of wildcelery that attracted great numbers of canvasback, red head, and scaup.

Dewey (1986) believed that although direct effects of atrazine for some aquatic insects may occur at concentrations as low as 20 ug/l, primary impacts to the insect community were probably indirect, a consequence of reduced food and habitat. At 20 ug/l significant reductions of aquatic insect abundance and species richness occurred. This could pose a concern to those managing waterfowl areas because of the importance of invertebrates in the spring diet of waterfowl. The atrazine levels measured in northwestern Ohio waters in 1991 were well below those likely to have such effects, but peak levels in previous years have far exceeded 20 ug/l.

Buhl and Faerber (1989) did acute toxicity tests on midges (chironomids) that showed relatively high concentrations of alachlor were necessary to cause death (see Table 3). In tests of 8 herbicides (not including atrazine), alachlor was among the group that, unlike the others, displayed toxicity increase with increased exposure duration. In a critical runoff event alachlor was among the group of herbicides that posed the greatest direct threat to midges and other wetland biota of similar sensitivity (Buhl and Faerber 1989). The literature contains little information on the toxicity of

alachlor to aquatic plants.

No standardized test protocols have been developed for the protection of submerged aquatic macrophytes (Fleming et al. 1991), and conditions under which toxicity tests with aquatic macrophytes are conducted appear to greatly influence test results. Canadian water quality guidelines for atrazine are 2 ug/l for aquatic life protection, 10 ug/l for irrigation water, and 60 ug/l for drinking water. The aquatic life protection guideline was intended to protect vascular plants and algae (Bodo 1991). Half life estimates for atrazine in surface waters are highly variable from 3 days to eight months (Bodo 1991).

In 1991 triazine concentrations of northwest Ohio streams and Lake Erie often exceeded the 2 ug/l Canadian guideline in the months of June and July. The highest triazine concentration recorded in the present study of northwestern Ohio was 4.67 ug/l which occurred in Muddy Creek on June 17, 1991. Ottawa NWR pools and moist soil units at no time in the sampling period exceeded the Canadian 2 ug/l guideline.

The triazine concentrations in the waters of northwestern Ohio in 1991 seldom reached levels that could have toxic effects on most biota (see Table 4), they were at the low end of the range where obvious environmental effects would be expected. Alachlor and related herbicide levels were significantly higher than triazines but there was little in the literature to indicate what environmental effects, if any, could be expected. There did not appear to be many studies in the literature investigating the type of pulsed exposure to which aquatic biota are subject in the field. Several authors were of the opinion that ecosystem toxic effects of herbicides in surface water were minimal and short lived and that aquatic biota would rapidly recover. Krieger et al. (1988) believed that increased herbicides uptake related to storm events would be a less important cause of stress to aufwuchs communities than factors like low temperatures, increased turbidity, and scouring.

On Ottawa NWR the concentrations of both the triazines and alachlor related herbicides in 1991 were lower than in other area waters and probably posed no toxic problem. The unmeasured peak concentrations in streams and rivers following storms are more problematic. The literature reviewed for this study contained relatively little information on the toxicity of alachlor to aquatic organism, especially plants. The great difference in alachlor toxicity between species, such as Lemna and Potamogeton (see Table 3), and the general lack of toxicity information for alachlor, as well as many other herbicides, make interpretation of environmental concentrations difficult.

Results of ELISA analyses for triazine and alachlor herbicides in waters of northwestern Ohio in 1991 fit within known patterns and magnitudes of concentrations determined by other analytical methods. ELISA analyses are well within the capability of Service field staff and offer an inexpensive alternative where analysis of large numbers of samples is required for a known compound. In 1991 the concentrations of triazines and alachlor and related herbicides in waters of Ottawa NWR were much lower than in other area waters and probably posed little risk to biota. Further laboratory research would be necessary to properly characterize the effects of waterborne herbicides on wetlands and other aquatic systems.

There appears to be only a short period of time between precipitation and associated river flow increases (see Figs. 2 and 3). Flow peaks associated with rain appear to last 10 to 15 days (see Fig. 3). Because storm runoff contains elevated concentrations of herbicides, refuge managers should maintain an awareness of precipitation for the 10 days previous to pumping water into refuge units. If pumping could be scheduled when there had been little previous rain then the concentration of herbicides in the water should be low.

Table 3. Critical concentrations of alachlor (exposure times not included) for components of aquatic ecosystems. This table is meant to give an indication of relative sensitivities to alachlor.

AFFECTED AQUATIC SYSTEM	ALACHLOR CONCENTRATION	INVESTIGATOR
Lemna minor growth EC50	10.1 - 14.5 ug/l	Hartman & Martin 1985
Potamogeton pectinatus growth no effect	1,000 ug/l	Hartman & Martin 1985
Potamogeton pectinatus growth reduction	10,000 ug/l	Hartman & Martin 1985
Chironomus riparius acute toxicity EC50	10,000 - 12,000 ug/l	Buhl & Faerber 1989
Chironomus plumosus acute toxicity EC50	2,500 - 3,200 ug/l	Buhl & Faerber 1989
Daphnia magna acute toxicity EC50	7,700 - 35,000 ug/l	Buhl & Faerber 1989
Rainbow trout acute toxicity EC50	1,400 - 4,200 ug/l	Buhl & Faerber 1989
Bluegill acute toxicity EC50	2,800 - 6,400 ug/l	Buhl & Faerber 1989

Table 4. Critical concentrations of atrazine (exposure times not included) for components of aquatic ecosystems. This table is only meant to give an indication of relative sensitivities to atrazine.

AFFECTED AQUATIC SYSTEM	ATRAZINE CONCENTRATION	INVESTIGATOR
Cyanobacteria disappear	100 ug/l	Herman et al. 1986
Reduced periphyton biomass and species composition	1 mg/kg	Kosinsky 1984
Primary producers affected	50 ug/l	Brockway et al. 1984
Nitrifying bacteria nitrogen metabolism	1,000 ug/l	Gadkari 1988
LOEC O, Mg, and Ca metabolism in microbes	32 ug/l	Pratt et al. 1988
Protozoan species richness reduced	337 ug/l	Pratt et al. 1988
Protozoan colonization rate reduced	3.2 ug/l	Pratt et al. 1988
Valisneria americana growth reduced	3.2 - 12 ug/l	Correl & Wu 1982
LOEC in Leffler microcosm (algae & invertebrates)	100 ug/l	Stay et al. 1989
Growth of Lemna minor reduced	100 ug/l	Hartman & Martin 1985
Growth of Potamogeton pectinatus reduced	100 ug/l	Hartman & Martin 1985
Brook trout fry growth reduced	120 ug/l	Macek et al. 1976
Gammarus fasciatus NOEL	490 ug/l	Macek et al. 1976
Gammarus fasciatus survival reduced	940 ug/l	Macek et al. 1976
Chara sp. production	100 ug/l	Dewey 1986
Chironomid emergence reduced	20 ug/l	Dewey 1986
Najas sp. growth reduced	20 ug/l	Dewey 1986
Potamogeton spp. growth reduced	20 ug/l	Dewey 1986
Periphyton growth reduced	100 ug/l	Dewey 1986
Aquatic insect species richness and abundance reduced	20 ug/l	Dewey 1986
Elodea sp. growth EC50	80 ug/l	Forney & Davis 1981
Myriophyllum sp. growth EC50	1,000 ug/l	Forney & Davis 1981

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